I hereby certify that this paper is being deposited in the United States Postal Service as first class mail in an envelope addressed to the Commissioner of Patents and Trademarks, Washington, D.C. 20231 on December 22, 1994.

Barbara G. McClung 12/22/94

Barbara G. McClung Date
Reg. No. 33,113

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: VAN NEST, et al.

Serial No.: 08/215,007 Group: 1811

Filed: March 21, 1994 Examiner: C. Salata

For: ADJUVANT FORMULATION COMPRISING A SUBMICRON OIL DROPLET EMULSION

DECLARATION UNDER 37 C.F.R. 1.132

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

1. We, Gary A. Van Nest, of 4890 San Pablo Dam Road, El Sobrante, California, and Gary Ott, of 112 Marlow Drive, Oakland, California, and Gail L. Barchfeld, of 2225 Romey Lane, Hayward, California, do swear that we are co-inventors of the above-captioned patent application, Serial No. 08/215,007.

2. Furthermore, we have read the Office Action, dated June 27, 1994, and are familiar with Cantrell, et al., U.S. 4,803,070, and Glass, et al., U.S. 3,919,411.

3. In our laboratories, we performed the experiments summarized in Figure 1 hereto. These animal studies showed the effect on mean antibody titers of various adjuvants combined with HIV gp120 antigen. Specifically, the following adjuvants were tested in baboons: alum (a suspension of aluminum hydroxide particles in water and the only adjuvant approved for human use), a Cantrell oil-in-water emulsion obtained from Ribi ImmunoChem Research Inc., and two of our submicron oil-in water emulsions. As can be seen, the submicron emulsions generated unexpectedly higher antibody titers.

4. After the Examiners' Interview on June 2, 1994, we undertook to determine the size of the oil droplets obtained by following the method described in Cantrell, et al., U.S. 4,803,070 Our procedure was as follows: 10 mg Ribi monophosphoryl lipid A (MPL referred to as refined detoxified endotoxin in U.S. 4,803,070) was dissolved in 1 ml 4:1 chloroform/methanol and 0.5 ml was transferred to a 15 ml Wheaton glass dounce homogenizer. 10 mg trehalose dimycolate (TDM) was dissolved in 1 ml 4:1 chloroform:methanol which was combined with the MPL solution in the dounce. Solvent was blown off with a stream of dry nitrogen. 2 ml squalene (Sigma) was added to the dounce dissolving the MPL and TDM. 98 ml of 0.2% Tween 80 was made by stirring 0.2 ml Tween into 98 ml PBS (0.15 M NaCl, .1 M sodium phosphate pH=7.4). 10 ml of 0.25 Tween was added to the dounce and a pre-emulsion made by five passes of a Type A (tight-fitting) pestle. The pre-emulsion was combined with the remaining 0.2% Tween 80 and transferred to a 100 ml homogenizing cylinder which was fitted to the Yamato LH21 homogenizer. The emulsion was homogenized with the Teflon pestle supplied by the manufacturer at 100 RPM for five minutes. Emulsion size was determined by laser light-scattering in the Malvern Mastersizer X using the lens system suitable for size determination in the 0.1-80 u range.

5. The Ribi emulsion (Cantrell) had a volume averaged mean diameter of 22.4 u (see D[4 3] on the chart, see Figure 2a. A control submicron oil-in-water emulsion of ours had a volume averaged mean diameter of .36 u, see Figure 2b. Thus, the emulsion described by Cantrell is significantly larger than our claimed submicron emulsions.

6. We declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereof.

Date: 12/22/94

Gary A. Van Nest

Date: 12/22/94

Gary Ott

Date: 12/22/94

Gail L. Barchfeld

I hereby certify that this paper is being deposited in the United States Postal Service as first class mail in an envelope addressed to the Assistant Commissioner of Patents, Washington, D.C. 20231-0001 on May 14, 1997.

Barbara G. McClung 5/14/97
Barbara G. McClung Date
Reg. No. 33,113

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Gary Van Nest, et al.	
Serial No.:	08/418,870	Group: 1813
Filed:	April 7, 1995	Examiner: H. Auer
For:	ADJUVANT FORMULATION COMPRISING SUBMICRON OIL DROPLET EMULSION	

DECLARATION UNDER 37 C.F.R. 1.132

Assistant Commissioner of Patents
Washington, D.C. 20231-0001

Sir:

1. We, Gary A. Van Nest, of 4890 San Pablo Road, El Sobrante, California; Gary Ott, of 112 Marlow Drive, Oakland, California; and Gail L. Barchfeld, of 2225 Romey Lane, Hayward, California, do swear that we are co-inventors of the above-captioned patent application, Serial No. 08/418,870.

2. In our laboratories, we performed the experiments summarized in paragraphs 3, 4, and 5 below. These data clearly demonstrate that our submmicron oil-in-water adjuvant compositions can have a potent adjuvant activity even when delivered to a site remote form the site of antigen delivery. This adjuvant activity could not be associated with any antigen depot effect.

3. Materials and Methods: Groups of 10 New Zealand White rabbits were used. One group of animals was injected with 25 µg of recombinant gD2 from herpes simplex virus (HSV) without adjuvant in the thigh muscle. A second group of rabbits was injected almost simultaneously with 25 µg of gD2 without adjuvant in one thigh and with 0.25 ml of "MF59" adjuvant (a submicron oil-in-water adjuvant composition having 5% squalene (v/v), 0.5% polysorbitan 80, 0.5% sorbitan trioleate, in citrate buffer) in the opposite thigh. Booster immunizations identical to the primary immunizations were given 21 days later. 14 days after each immunization, animals were bled and anti-gD2 antibody titers were determined by enzyme linked immunoadsorbant assay.

4. The antibody results are shown:

Group	Rabbit Number	Anti-gD2 titer 14 days post 1st	Anti-gd2 titer 14 days post 2nd
1 gD2 without adjuvant	497 498 499 500 501 502 503 504 505 506 geometric mean ± standard error	31 16 5 23 17 33 21 500 13 13 24 ± 9	847 1524 67 600 126 1310 43 963 320 51 300 ± 133
2 gD2 in one thigh MF59 in opposite thigh	487 488 489 490 491 492 493 494 495 496 geometric mean ± standard error	34 27 595 44 97 35 88 12 583 17 62 ± 26	9216 4001 29115 5868 8173 4636 6797 1004 4433 546 4596 ± 1229

5. Conclusions: After one immunization, MF59 delivered in the opposite thigh was able to stimulate antibody titers to gD2 approximately three-fold. After two immunizations, MF59 delivered in the opposite thigh stimulated titer approximately 15-fold.

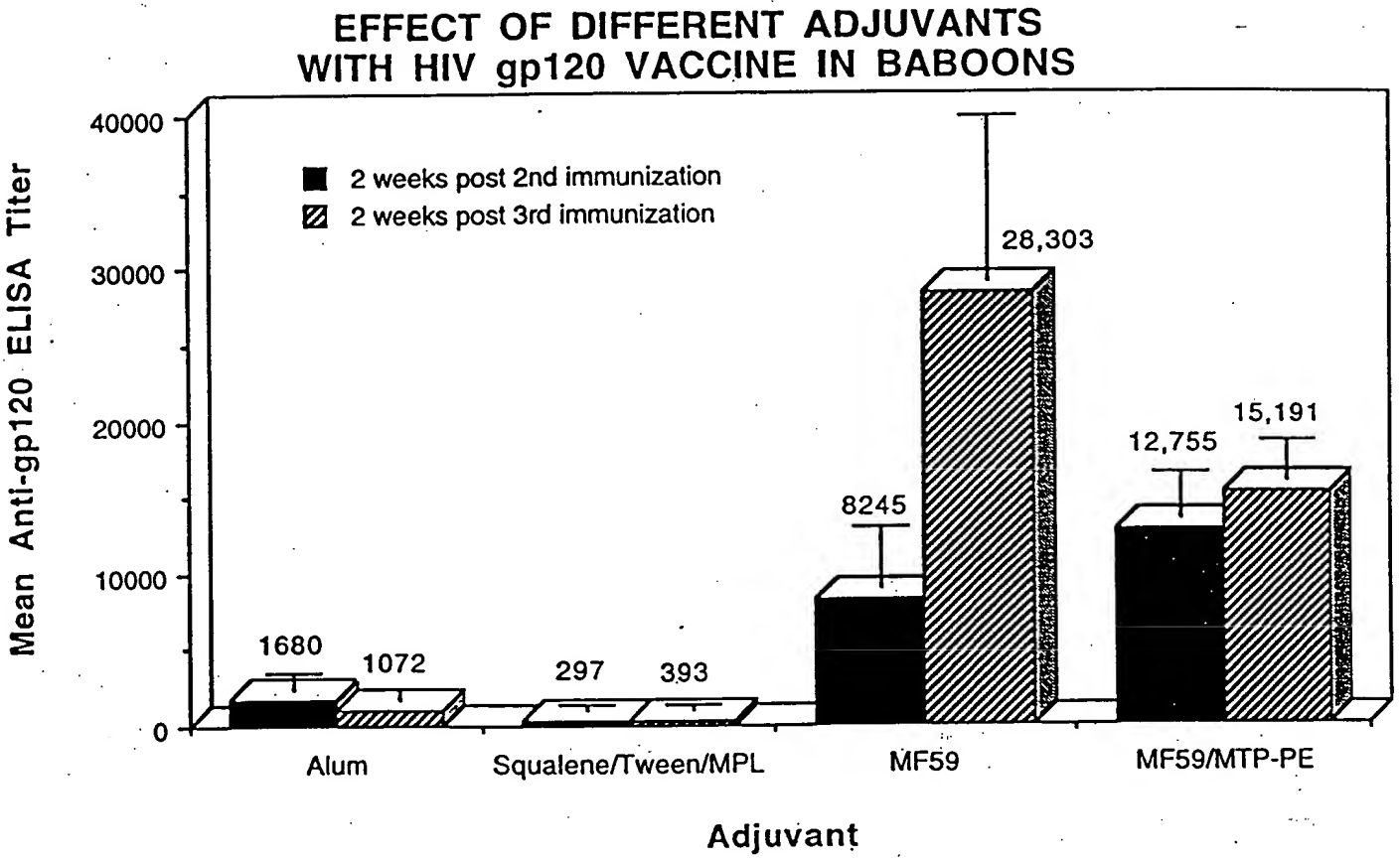
6. We declare that all statements made herein of our knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereof.

Date: May 14, 1997 — Gary A. Van Nest

Date: May 14, 1997 — Gary Ott

Date: May 14, 1997 — Gail L. Barchfeld

FIGURE 1



Groups of five baboons were immunized three times (at week 0, week 8, and week 24) with 50 μg of gp120 and the different adjuvants. Two weeks after the second and third immunizations, animals were bled and anti-gp120 antibody titers were determined by ELISA. The values expressed are the geometric means titers ± standard error for the adjuvant groups. The adjuvants used include alum (aluminum hydroxide), squalene/Tween 80/monophosphoryl lipid A (Cantrell formulation prepared by Ribi Immunochem, Inc,), MF59 (microfluidized emulsion containing 5% squalene, 0.5% Tween 80, and 0.5% Span 80), and MF59/MTP-PE (microfluidized emulsion containing 5% squalene, 0.5% Tween 80, 0.5% Span 85 and 50μg MTP-PE).

Figure 2a

MALVERN MASTERSIZER X

Version 1.2b

Wed, Aug 03, 1994 9:39AM

Gary's RIBI :Run Number 1

8/3/94

Sample File Name: TEST , Record: 143
Measured on: Wed, Aug 03, 1994 9:39AM Last saved on: Wed, Aug 03, 1994 9:39AM

Source: Analysed

Presentation: 20HD
Very Polydisperse model

Volume Result

Residual = 0.462 %
d (0.5) = 17.75 µm
D [4, 3] = 22.41 µm
Sauter Mean (D[3,2]) = 3.40 µm
Specific Surface Area = 1.7656 sq. m. / gm

Concentration = 0.009 %
d (0.1) = 1.17 µm
Span = 2.98

Focus = 45 mm.

Obscuration = 14.75 %
d (0.9) = 54.09 µm

Mode = 29.89 µm
Density = 1.00 gm. / c.c.

Size (Lo) µm	Result In %	Size (Hi) µm	Result Below %
0.05	0.03	0.12	0.03
0.12	0.05	0.15	0.08
0.15	0.08	0.19	0.16
0.19	0.15	0.23	0.32
0.23	0.25	0.28	0.57
0.28	0.40	0.35	0.97
0.35	0.60	0.43	1.57
0.43	0.89	0.53	2.46
0.53	1.27	0.65	3.72
0.65	1.75	0.81	5.47
0.81	2.33	1.00	7.81
1.00	2.95	1.23	10.76
1.23	3.39	1.51	14.14
1.51	3.45	1.86	17.59
1.86	3.07	2.30	20.66
2.30	2.52	2.83	23.18

Size (Lo) µm	Result In %	Size (Hi) µm	Result Below %
2.83	2.02	3.49	25.19
3.49	1.75	4.30	26.94
4.30	1.66	5.29	28.60
5.29	1.81	6.52	30.42
6.52	2.16	8.04	32.57
8.04	2.88	9.91	35.45
9.91	3.87	12.21	39.32
12.21	5.45	15.04	44.77
15.04	6.75	18.54	51.52
18.54	7.86	22.84	59.38
22.84	7.94	28.15	67.32
28.15	7.96	34.69	75.28
34.69	7.30	42.75	82.58
42.75	6.64	52.68	89.22
52.68	5.71	64.92	94.94
64.92	5.06	80.00	100.00

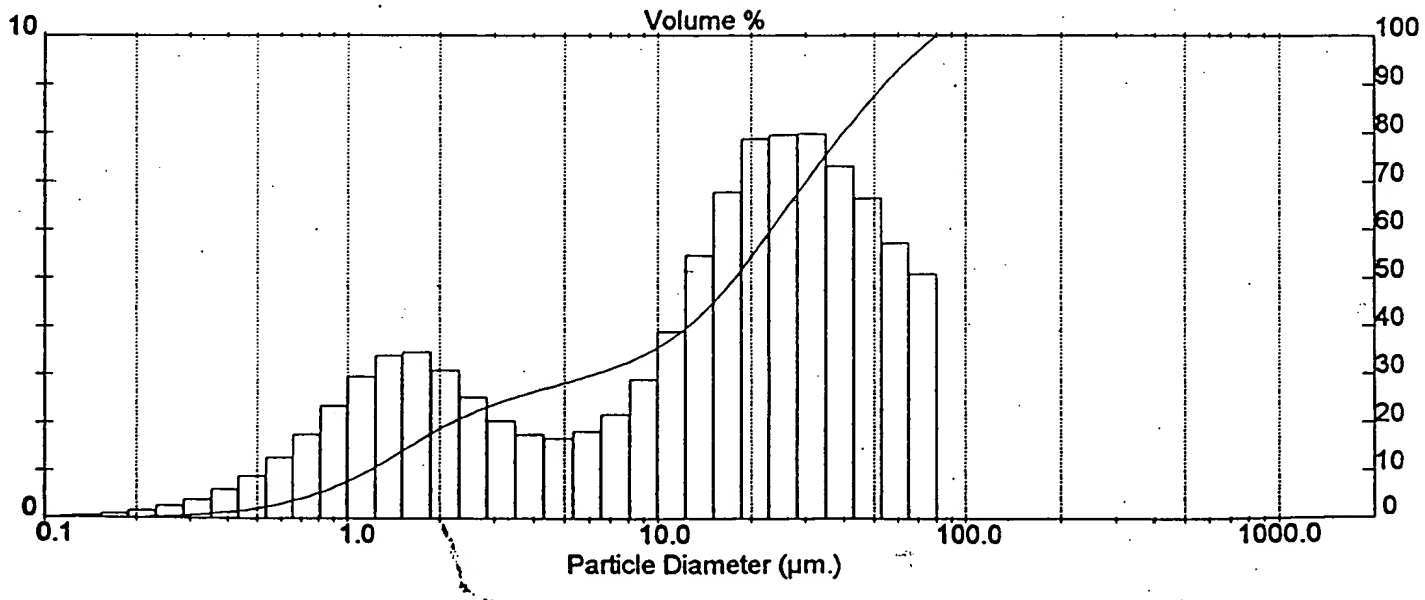


Figure 2b

MALVERN MASTERSIZER X

Version 1.2b

Wed, Aug 03, 1994 9:52AM

MF59-0, bottle 2 :Run Number 3

8/3/94

Sample File Name: TEST , Record: 145
Measured on: Wed, Aug 03, 1994 9:52AM Last saved on: Wed, Aug 03, 1994 9:52AM

Source: Analysed

Presentation: 2OHD
Very Polydisperse model

Volume Result

Focus = 45 mm.

Residual = 2.325 %
d (0.5) = 0.33 µm
D [4, 3] = 0.36 µm
Sauter Mean (D[3,2]) = 0.30 µm
Specific Surface Area = 20.1342 sq. m. / gm

Concentration = 0.002 %
d (0.1) = 0.19 µm
Span = 1.13

Obscuration = 13.71 %
d (0.9) = 0.56 µm

Mode = 0.32 µm
Density = 1.00 gm. / c.c.

Size (Lo) µm	Result In %	Size (Hi) µm	Result Below %
0.05	0.85	0.12	0.85
0.12	1.33	0.15	2.18
0.15	6.87	0.19	9.05
0.19	11.70	0.23	20.75
0.23	16.45	0.28	37.20
0.28	19.25	0.35	56.45
0.35	17.91	0.43	74.36
0.43	13.33	0.53	87.69
0.53	7.69	0.65	95.38
0.65	3.19	0.81	98.57
0.81	0.98	1.00	99.55
1.00	0.27	1.23	99.82
1.23	0.09	1.51	99.91
1.51	0.04	1.86	99.95
1.86	0.02	2.30	99.97
2.30	0.01	2.83	99.98

Size (Lo) µm	Result In %	Size (Hi) µm	Result Below %
2.83	0.01	3.49	99.99
3.49	0.01	4.30	99.99
4.30	0.00	5.29	100.00
5.29	0.00	6.52	100.00
6.52	0.00	8.04	100.00
8.04	0.00	9.91	100.00
9.91	0.00	12.21	100.00
12.21	0.00	15.04	100.00
15.04	0.00	18.54	100.00
18.54	0.00	22.84	100.00
22.84	0.00	28.15	100.00
28.15	0.00	34.69	100.00
34.69	0.00	42.75	100.00
42.75	0.00	52.68	100.00
52.68	0.00	64.92	100.00
64.92	0.00	80.00	100.00